IN THE SPECIFICATION

Please replace paragraph [0004] with the following:

[0004] Human bones are always remodelling by the repeated process of resorption and reconstitution. Osteoblasts and osteoclasts are considered to be the cells mainly responsible for bone formation and bone resorption, respectively. A typical example of a disease caused by abnormal bone metabolism is osteoporosis. Osteoporosis is known to ~~result~~ develop when bone resorption by osteoclasts exceeds bone formation by osteoblasts, but the mechanism of osteoporosis has not yet been completely elucidated. Osteoporosis causes bone pain and makes bones fragile, leading to fracture, particularly in elderly patients. Osteoporosis has therefore become a social issue with the increasing number of elderly people in the population. Therefore, effective drugs for the treatment of the disease are expected to be developed. Bone mass reduction caused by abnormal bone metabolism is thought to be prevented by inhibiting bone resorption, improving bone formation, or improving the balance of bone metabolism.

Please replace paragraph [0007] with the following:

[0007] These cytokines are expected to be effective drugs for improving bone mass reduction by stimulating bone formation and/or by inhibiting bone resorption. Cytokines such as insulin-like growth factor-I and bone morphogenetic proteins have been investigated in clinical trials for their effectiveness for treating patients with bone diseases. Calcitonin is already used ~~to treat~~ for osteoporosis and to diminish pain in osteoporosis patients.

Please replace the paragraph beginning on page 7, line 3, with the following:

[0008] Examples of drugs now clinically utilized for the treatment of bone diseases and for shortening the treatment period are dihydroxyvitamine D_3, vitamin K_2, calcitonin and its derivatives, hormones such as estradiol, ipriflavon, and calcium preparations. However, these drugs do not provide satisfactory therapeutic effects, and novel drug substances are expected to be developed. Since bone metabolism is manifest in the balance between bone resorption and

bone ~~formulation~~ formation, cytokines which inhibit osteoclast differentiation and/or maturation are expected to be developed as drugs for the treatment of bone diseases such as osteoporosis.

Please replace paragraph [0014] with the following:

[0014] The invention includes a method for purifying OCIF protein, comprising: (1) culturing human fibroblasts, (2) applying the conditioned medium to a heparin column to obtain the adsorbed fraction, (3) purifying the OCIF protein using a cation-exchange column, (4) purifying the OCIF protein using a heparin affinity column, (5) purifying the OCIF protein using a ~~Cibachrome~~ Cibacron blue affinity column, and (6) isolating the OCIF protein using reverse-phase column chromatography. ~~Cibachrome~~ Cibacron blue F3GA dye may be coupled to a carrier made of synthetic hydrophilic polymers, for example, to form columns conventionally called "blue columns".

Please replace paragraph [0033] with the following:

[0033] The OCIF protein of the instant invention can be obtained initially as a basic heparin binding OCIF fraction by applying the culture medium to a heparin column (Heparin-SEPHAROSE™ CL-6B, Pharmacia), eluting with 10 mM Tris-HCl buffer, pH 7.5, containing 2 M NaCl, and applying the OCIF fraction to a Q • anion-exchange column (HILOAD™-Q/FF, Pharmacia), and collecting the non-adsorbed fraction. OCIF protein can be purified by subjecting the obtained OCIF fraction to purification on an S • cation-exchange column (HILOAD™-S/FF, Pharmacia), a heparin column (Heparin-5PW, TOSOH), a ~~Cibachrome~~ Cibacron Blue column (Blue-5PW, TOSOH), and a reverse-phase column (BU-300 C4, Perkin Elmer).

Please replace paragraph [0036] with the following:

[0036] The present invention further includes OCIF mutants. They are substitution mutants comprising the replacement of one cysteine residue, possibly involved in dimer formation, with a serine residue or various deletion mutants of OCIF. Substitutions or deletions are introduced into

the OCIF cDNA using polymerase chain reaction (PCR) or restriction enzyme digestion. Each of these mutated OCIF cDNAs is inserted into a vector having an appropriate promoter for gene expression. The resultant expression vector for each of the OCIF mutants is ~~transfected~~ introduced into eukaryotic cells such as mammalian cells. Each of OCIF mutants can be obtained and purified from the conditioned media of the transfected cells.

Please replace paragraph [0045] with the following:

[0045] The OCIF protein of the invention is useful as a pharmaceutical ingredient for treating or improving decreased bone mass in bone diseases such as osteoporosis, rheumatism, osteoarthritis, and abnormal bone metabolism in multiple myeloma. OCIF protein is also useful as an antigen in the immunological diagnosis of bone diseases. Pharmaceutical preparations containing OCIF protein as an active ingredient are formulated and can be orally or parenterally administered. The preparation contains the OCIF protein of the present invention as an effective ingredient and is safely administered to humans and animals. Examples of pharmaceutical preparations include compositions for injection or intravenous drip, suppositories, nasal preparations, sublingual preparations, and tapes for percutaneous absorption. The pharmaceutical preparation for injection can be prepared by mixing a pharmacologically effective amount of OCIF protein and a pharmaceutically acceptable carrier. The carriers are vehicles and/or activators, e.g. amino acids, saccharides, cellulose derivatives, and other organic and inorganic compounds, which are generally added to active ingredients. When the OCIF protein is mixed with the vehicles and/or activators for injection, pH adjusters, buffers, stabilizers, solubilizing ~~agent~~ agents, etc. can be added by conventional methods, if necessary.

Please replace paragraph [0070] with the following:

[0070] Each 2 fractions (1 ml) from fractions 51 to 70 of the blue-5PW fractions were acidified with 10 μL of 25% TFA, and applied to a reverse phase C4 column (BU-300, 2.1 x 220 mm, manufactured by Perkin-Elmer Co.) equilibrated with 25% acetonitrile containing 0.1% TFA. The adsorbed protein was eluted with a 12 ml linear gradient of 25 to 55% acetonitrile at a flow

rate of 0.2 ml/min, and the protein fractions corresponding to peaks 6 and 7 were collected, respectively. The protein from each peak was applied to a protein sequencer (PROCISE™ 494, Perkin-Elmer Co.). However, the N-terminal sequence of the ~~proteins~~ protein of each peak could not be analyzed. Therefore, the N-terminus of the protein of each peak was considered to be blocked. Internal amino acid sequences of these proteins were therefore analyzed.

Please replace paragraph [0121] with the following:

[0121] Each of the rOCIF(E) and nOCIF samples were diluted with α-MEM™ (GIBCO BRL Co.) containing 10% FBS and 2 x 10^{-8} M of activated vitamin D_3 (a final concentration of 250 ng/ml). Each sample was serially diluted with the same medium, and 100 μL of each diluted sample was added to each well of a 96-well plate. Bone marrow cells obtained from 17 day old mice were inoculated at a cell density of 3 x 10^5 cells/100 μL/well into each well of a 96-well plate and cultured for 7 days at 37°C in humidified 5% CO_2. On day 7, the cells were fixed and stained with an acid phosphatase measuring kit (Acid Phosphatase, Leucocyte, No. 387-A, Sigma) according to the method described in EXAMPLE 2. A decrease in acid phosphatase activity (TRAP) was taken as an indication of OCIF activity. A decrease in acid phosphatase-positive cells was evaluated by solubilizing the pigment of dye and measuring absorbance. Briefly, 100 μL of a mixture of 0.1 N NaOH and dimethylsulfoxide (1:1) was added to each well and the well was vibrated to solubilize the dye. After solubilizing the dye completely, an absorbance of each well was measured at 590 nm, subtracting the absorbance at 490 nm using a microplate reader (IMMUNOREADER™ NJ-2000, InterMed). The microplate reader was adjusted to 0 absorbance using a well with monolayered bone marrow cells which was cultured in the medium without activated vitamin D_3. A decrease in TRAP activity was expressed as a percentage of the control absorbance value (=100%) (~~the solubilized dye from~~ measured for wells with bone marrow cells cultured in the absence of OCIF). The results are shown in Table 5.

Please replace paragraph [0134] with the following:

[0134] Each rOCIF(E) and rOCIF(C) sample containing 100 μg of OCIF protein, was supplemented with 1/100 volume of 25% trifluoro acetic acid and applied to a reverse phase column (~~Protein-RP~~ PROTEIN-RP™, 2.0 x 250 mm, YMC Co.) equilibrated with 30% acetonitrile containing 0.1% trifluoro acetic acid. OCIF protein was eluted from the column with a linear gradient from 30 to 55% acetonitrile at a flow rate of 0.2 ml/min for 50 min. and each OCIF peak was collected. The monomer-type OCIF peak fraction and dimer-type OCIF peak fraction were each lyophilized.

Please replace paragraph [0142] with the following:

[0142] The plasmid pBKOCIF, comprising OCIF cDNA inserted into plasmid pBKCMV (Stratagene), was obtained as in EXAMPLES 10 and 11. Further, during the screening of the cDNA library with the 397 bp OCIF cDNA probe, the transformants containing plasmids whose insert sizes were different from that of pBKOCIF were obtained. These transformants containing the plasmids were grown and the plasmids were purified according to the standard method. The sequence of the insert DNA in each plasmid was determined using a TAQ DYE DEOXY TERMINATER CYCLE SEQUENCING™ kit (Perkin Elmer). The primers used were T3, T7, (Stratagene) and synthetic primers prepared based on the nucleotide sequence of OCIF cDNA. There are four OCIF variants (OCIF2, 3, 4, and 5) in addition to OCIF. The nucleotide sequence of OCIF2 is shown in SEQ ID NO: 8 and the amino acid sequence of ~~OCIF-2~~ OCIF2 predicted by the nucleotide sequence is shown in SEQ ID NO: 9. The nucleotide sequence of OCIF3 is shown in SEQ ID NO: 10 and the amino acid sequence of OCIF3 predicted by the nucleotide sequence is shown in SEQ ID NO: 11. The nucleotide sequence of OCIF4 is shown in SEQ ID NO: 12 and the amino acid sequence of OCIF4 predicted by the nucleotide sequence is shown in SEQ ID NO: 13. The nucleotide sequence of OCIF5 is shown in SEQ ID NO: 14 and the amino acid sequence of OCIF5 predicted by the nucleotide sequence is shown in SEQ ID NO: 15. The structures of OCIF variants are shown in Figures 9 to 12 and are briefly described below.

Please replace paragraph [0221] with the following:

[0221] Ten microliters of the final conditioned medium was used for western blot analysis. Ten microliters of each sample were mixed with 10 μL of SDS-PAGE sample buffer (0.5 M Tris-HCl, 20% glycerol, 4% SDS, 20 μg/ml bromophenol blue, pH 6.8), boiled for 3 min. and subjected to 10% SDS polyacrylamide gel electrophoresis under non-reducing conditions. After the electrophoresis, the separated proteins were blotted to PVDF membrane (PROBLOTT™, Perkin Elmer) using a semi-dry electroblotter (BIO-RAD). The membrane was incubated at 37°C with horseradish peroxidase-labeled anti-OCIF antibodies for 2 hr. After the membrane was washed, protein bands which react with the labeled antibodies were detected using an ECL™ system (Amersham). Two protein bands with approximate molecular ~~masses~~ weights of 60 kD and 120 kD were detected for the unaltered OCIF. On the other hand, almost exclusively a 60 kD protein band was detected for the OCIF-C23S, OCIF-CL and OCIF-CC mutants. Protein bands with approximate ~~masses~~ weights of 40 kD-50 kD and 30 kD-40 kD were the major ones for OCIF-CDD2 and OCIF-CDD1, respectively. These results indicate that Cys at 379 is responsible for the dimer formation, both the monomers and the dimers maintain the biological activity and a deletion of residues from Asp at 177 to Leu at 380 does not abolish the biological activity of OCIF (positions of the amino acid residues are shown in SEQ ID NO: 4).

Please replace paragraph [0225] with the following:

[0225] The library was titered, and 1 x 10^6 pfu of phage was mixed with XL1-Blue MRA host *E. coli* cells and plated onto 20 plates (9 cm x 13 cm) with 9 ml per plate of top agarose. The plates were incubated overnight at 37°C. Filter plaque lifts were prepared using HYBOND™-N nylon membranes (Amersham). The membranes were processed by denaturation in a solution containing 1.5 M NaCl and 0.5 M NaOH for 1 minute at room temperature. The membranes were then neutralized by placing each one in 1 M Tris-HCl (pH 7.5) and a solution containing 1.5 M NaCl and 0.5 M Tris-HCl (pH 7.5) ~~successfully~~ successively for one minute each. The membranes were then transferred onto a filter paper wetted with 2 x SSC. Phage DNA was fixed onto the membranes with 1200 microJoules of UV energy using a STRATALINKER UV

CROSSLINKER™ 2400 (STRATAGENE) and the membranes were air dried. The membranes were immersed in Rapid Hybridization buffer (Amersham) and incubated for one hour at 65°C before hybridization with ^{32}P-labeled cDNA probe in the same buffer overnight at 65°C. Screening probe was prepared by labeling the OCIF cDNA with ^{32}P using the Megaprime DNA labeling system (Amersham). Approximately, 5 x 10^5 cpm probe was used for each ml of hybridization buffer. After the hybridization, the membranes were rinsed in 2 x SSC for five minutes at room temperature. The membranes were then washed four times, 20 minutes each time, in 0.5 x SSC containing 0.1% SDS at 65°C. After the final wash, the membranes were dried and subjected to autoradiography at -80°C with SUPER HR-H™ X-ray film (FUJI PHOTO FILM Co., Ltd.) and an intensifying screen.

Please replace paragraph [0258] with the following:

[0258] Six week old male Fischer rats were subjected to denervation of the left forelimb. These rats were assigned to four groups (10 rats/group) and treated as follows: group A, sham operated rats without administration; group B, ~~denervated~~ denerved rats with the vehicle administered intravenously; group C, ~~denervated~~ denerved rats with OCIF administered intravenously at a dose of 5 μg/kg twice a day; group D, ~~denervated~~ denerved rats with OCIF administered intravenously at a dose of 50 μg/kg twice a day. After denervation, OCIF was administered daily for 14 days. After 2 weeks treatment, the animals were sacrificed and their forelimbs were dissected. Thereafter bones were tested for mechanical strength.